IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Cerami *et al.*	Confirmation No.: 5726
Serial No.: 09/259,929	Art Unit: 1617
Filed: March 1, 1999	Examiner: Chong, Yong Soo
For: METHOD AND DEVICES FOR MODULATING THE IMMUNE RESPONSE	Attorney Docket No: 10162-004-999

DECLARATION OF CARLA CERAMI HAND, PH.D., MD

Sir:

I, Carla Cerami Hand, do hereby declare and state:

1. I am a named inventor on the '929 application. I am presently Director of the Kenneth S. Warren Institute, Inc., a not-for –profit organization doing research into therapeutics for third world diseases and a Visiting Scholar at the School of Public Health at the University of North Carolina – Chapel Hill.

2. I have over 14 years of experience in biological research and clinical investigation. My academic and technical experience and honors, and a list of my publications, are set forth in my curriculum vitae, a copy of which is attached hereto as Appendix A.

3. I have read and am familiar with the '929 application, the pending claims and the outstanding Office Action. I understand that the technology of the '929 application relates to the use of a virtual lymph node device ("VLN") for inducing and enhancing an immune response to an antigen in a mammal. The VLN is an implantable device with a porous matrix containing an antigen surrounded by a perforated but otherwise impermeable container. The container on the device acts a diffusion barrier permitting the ingress and egress of immune cells but limiting the passive diffusion of antigens and co-stimulatory factors (cytokines, chemokines) from the device. I have been informed and believe that the claims of the '929 application are subject to a rejection based on the contention that the claims of '929 application are obvious in light of Barr et al. (US Patent No. 5,593,697) and Andrianov et al. (US Patent No. 5,529,777).

4. In the following paragraphs I will present the results of a clinical trial demonstrating the surprising effectiveness of the VLN in eliciting an immune response.

I. Clinical Study

5. In September 1999 a clinical trial entitled "Safety and Efficacy of a Subcutaneously Implanted Chamber for Inducing an Immune Response to Flu Vaccine" was initiated at Mount Sinai Medical Center.

6. The clinical trial was initiated to determine the effectiveness of the VLN in influenza vaccination. One of the problems with flu vaccines is that it does not stimulate the appropriate immune response. The flu vaccine will stimulate the production of antibodies, which will prevent the virus from taking hold in the body, but does not form killer immune cells, which would attack cell already infected with the influenza virus. In animal models, it was demonstrated that the VLN permitted immune cells to enter the device and mature into and propagate killer immune cells in addition to forming antibodies. Thus, it was thought that the VLN could generate the killer immune cells in a human vaccination.

7. The clinical trial consisted of five groups of healthy volunteers (6 per group): (1) a group receiving the VLN alone, (2) a group receiving the VLN containing 50 ng of FluShield (Connaught Laboratories, Inc.) influenza vaccine, (3) a group receiving the VLN containing 500 ng of FluShield, (4) a group receiving 500 ng of FluSheild, and (5) a group receiving 45 μg FluSheild. Blood samples (50 cc) were taken from each volunteer prior to immunization, and 14 and 28 days after immunization and analyzed for cell mediated immune response.

8. The VLN device was constructed in accordance with the disclosure of the '929 application and consisted of a one inch length of perforated silicone tubing (Specialty Manufacturing Inc.) filled with a polyvinyl alcohol sponge impregnated with FluSheild. The device was implanted subcutaneously in the volunteers.

9. The blood samples taken from the volunteers on Day 28 were tested for their cellular immune response to influenza using a cytotxic T-lymphocyte assay. The blood cells were cultured to increase the number of cytotoxic T-lymphocytes. On the day of

the experiment, the cell cultures were split and one half of the culture was infected with the influenza virus and labeled with Chromium-51. The cell cultures were then mixed together.

10. If the cytotoxic T-lymphocytes recognize the virus-infected cells, Chomium is released in to the media.

II. Results of Clinical Trial

11. Attachment A is a graph illustrating the cytotoxic T-lymphocyte response to influenza infected cells from the blood cultures taken at 28 days from the volunteers: non-responders (those volunteers administered VLN without FluSheild, VLN with 50 nm FluSheild, and 500 nm FluSheild); those administered the VLN with 500 nm FluSheild; and those administered 45 μg FluSheild. The blood cultures from the volunteers that received the VLN and 500 ng of FluSheild had nearly a three fold increase in the cytotoxic T-lymphocyte response to infected cells in comparison to the blood cultures taken from volunteers receiving 45 μg of FluSheild intramuscularly. This response was particularly impressive given that the amount of FluSheild administered to the volunteers without the VLN was 90 times greater than the amount administered in the VLN.

III. CONCLUSION

12. In summary, I have presented data that demonstrate that the VLN helped induce a robust cellular immune response to influenza when administered to volunteers. This result was unexpected given that the amount of vaccine present in the VLN device was 90 times less than that administered to other volunteers that did not receive the VLN device and yet the response in the volunteers that received the VLN was nearly three times greater.

13. I declare further that all statements made in this Declaration of my own knowledge are true, that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully Submitted

8/13/08
Date

Carla Cerami Hand, Ph.D, M.D.

Attachments:

Appendix A: Curriculum Vitae of Dr. Carla Cerami Hand, Ph.D.

Appendix B: Clinical Trial: VLN Immunization Induces CTL Response (Day 28)

APPENDIX A

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME **Carla Cerami Hand**	POSITION TITLE **Visiting Scholar**
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Columbia University, New York, NY	BA	1987	Biochemistry
New York Univ. SOM, New York, NY	PhD	1993	Immunology
New York Univ. SOM, New York, NY	MD	1994	Medicine
North Shore Univ. Hospital, Manhasset, NY	Intern	1994-1995	Surgery

A. Positions and Honors

1995	Surgical Intern of the Year, North Shore University Hospital, Manhasset, NY
1995-1996	Staff Investigator, The Picower Institute for Medical Research, Manhasset, NY
1996-present	Member Scientist, The Kenneth S. Warren Institute, Ossining, NY
1996-present	Founder and Director, The Kenneth S. Warren Institute, Ossining, NY
2001-2004	Founder and Director, Warren Pharmaceuticals, Inc., New York
2002-2003	House Staff, Dept. of Pediatrics, Columbia University, New York, NY
2004	Chief Operating Officer, Warren Pharmaceuticals, Inc.
2007-Present	Visiting Scholar, University of North Carolina, School of Public Health, Chapel Hill, NC

Medical License

1996-present	New York State

B. Selected Peer-Reviewed Publications

1. **Cerami, C.**, Frevert, U., Sinnis, P., Takacs, B., Clavijo, P., Santos, M., Nussenzweig, V. (1992) The basolateral domain of the hepatocyte plasma membrane bears receptors for the circumsporozoite protein of Plasmodium falciparum sporozoites. *Cell* 70:1021-1033.
2. **Cerami, C.**, Kwakye-Berko, F., Nussenzweig, V. (1992) Binding of malarial circumsporozoite protein to sulfatides and cholesterol-3-sulfate: dependency on disulfide bond formation between cysteines in region II. *Molecular and Biochemical Parasitology* 54:1-12.
3. Frevert, U., Sinnis, P., Shreffler, **Cerami, C.,** W., Takacs, B., Nussenzweig, V. (1993) Malaria circumsporozoite protein binds to heparan sulfate proteoglycans associated with the surface membrane of hepatocytes. *Journal of Experimental Medicine* 177:1287-1298
4. Sinnis, P., Clavijo, P., Fenyo, D., Chaite, B., **Cerami, C.**, Nussenzweig, V. (1994) Structural and functional properties of region II-plus of the malaria circumsporozoite protein. *Journal of Experimental Medicine* 180:297-306.
5. **Cerami, C.**, Frevert, U., Sinnis, P., Takacs, B., Nussenzweig, V. (1994) Rapid clearance of malaria circumsporozoite protein by hepatocytes. *Journal of Experimental Medicine* 179:695-701.
6. **Cerami, C.**, Zhang, X., Ulrich, P., Bianchi, M., Tracey, K., Berger, B. (1995) High performance liquid chromatographic method for guanylhydrazone compounds. *Journal of Chromatography* B 675:71-82.
7. **Cerami, C.,** Founds, H., Nicholl, I., Mitsuhashi, T., Giordano, D., Vanpatten, S., Lee, A., Al-abed, Y., Vlassara, H., Bucala, R., Cerami, A. (1997) Tobacco smoke is a source of toxic reactive glycation products. *Proceedings of National Academy of Sciences* 94:13915-13920.
8. Brines, M.L., Ghezzi, P., Keenan, S., Agnello, D., de Lanerolle, N.C., **Cerami, C.,** Itri, L.M., Cerami, A. (2000) Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proceedings of National Academy of Sciences* 97(19): 10526-10531.
9. Erbayraktar S., Grasso G., Sfacteria A., Xie QW., Coleman T., Kreilgaard M., Torup L., Sager T., Erbayraktar Z., Gokmen L., Yilmaz O., Ghezzi P., Villa P., Fratelli M., Casagrande S., Leist M., Helboe L., Christensen S., Geist A., OStwegaard Pedersen L., **Cerami Hand C.,** Wuerth JP., Cerami A., Brines M.,

(2003) Asialoerythropoietin is a non-erythropoietin cytokine with broad neuroprotective activity in vivo, *Proceedings of National Academy of Sciences* Volume 100(11); 6741-6746.

10. Leist, M., Gehzzi, P., Grasso, G., Bianchi, R., Villa, P., Fratelli, M., Savino, C., Bianchi, M., Nielsen, J., Gerwein, J., Kallunki, P., Larsen, A., Helboe, L., Christensen, S., Pedersen, L., Nielsen, M., Torup, L., Sager, T., Sfacteria, A., Erbayraktar, S., Erbayraktar, Z., Gokemn, N., Yilmaz, O., **Cerami Hand, C.,** Xie, QW., Coleman, T., Cerami, A., Brines, M. (2004) Erythropoietin-derived tissue-protective cytokines that do not bind the classical erythropoietin receptor, *Science* Jul 9;305(5681):239-42.
11. Brines, M., Grasso, G., Fiordaliso, F., Sfacteria, A., Ghezzi, P., Latini, R., Xie, QW., Smart, J., Su-Rick CJ, Pobre, E., Diaz, D., Gomez, D., **Hand, C.**, Coleman, T., Cerami, A. (2004) Erythropoietin mediates tissue protection through an erythropoietin and common {beta}-subunit heteroreceptor *Proceedings of National Academy of Science* Volume 101(41):14907-12.
12. Wallach-Dayan, S.B., **Hand, C.**, Naor, D. (2006) CD44 Variant isoforms are required for experimental mammary tumor progression: protection by DNA vaccination (submitted, 2006)
13. Garin T, Rubinstein A, Grigoriadis N, Nedvetzki S, Abramsky O, Mizrachi-Koll R, **Hand C**, Naor D, Karussis D. (2007) CD44 variant DNA vaccination with virtual lymph node ameliorates experimental autoimmune encephalomyelitis through the induction of apoptosis. *Journal of Neurological Science*. 2007 Jul 15;258(1-2):17-26.
14. Wallach-Dayan SB , Rubinstein AM, **Hand C**, Breuer R, Naor D. DNA vaccination with CD44 variant isoform reduces mammary tumor local growth and lung metastasis. *Molecular Cancer Therapeutics*. 2008 Jun;7(6):1615-23.
15. Weiss L., Botero-Anug AM, **Hand C**, Slavin S, Naor D. CD44 gene vaccination for insulin-dependent diabetes mellitus in non-obese diabetic mice. *Israeli Medical Association Journal*. 2008 Jan;10(1):20-5.
16. Brines, M., Patel, N.S.A., Villa, P., Brines, C., Mennini, T., De Paola, M., Erbayraktar, Z., Erbayraktar, S., Sepodes, B., Thiemermann, Ghezzi, P., Yamin, M. **Hand, C.**, Xie, QW., Coleman, T., Cerami, A. Non-erythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. *Proceedings of National Academy of Science, in press.*

Reviews

1. Cerami, A., Brines, M., **Cerami, C.,** Ghezzi, P., (April 2001) Effects of Epoetin Alfa on the Central Nervous System - *"Seminars in Oncology"* Volume 28(2) Suppl. H pp 65-69.
2. Cerami, A., Brines, M., Ghezzi, P., **Cerami, C.,** Itri, L., (2002) Neuroprotective properties of Epoetin Alfa. *Nephrolology Dialysis, Transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association* - Supplemental 1:8-12.

US Patents and Patent Applications

(Each of the items listed below has associated international counterparts)

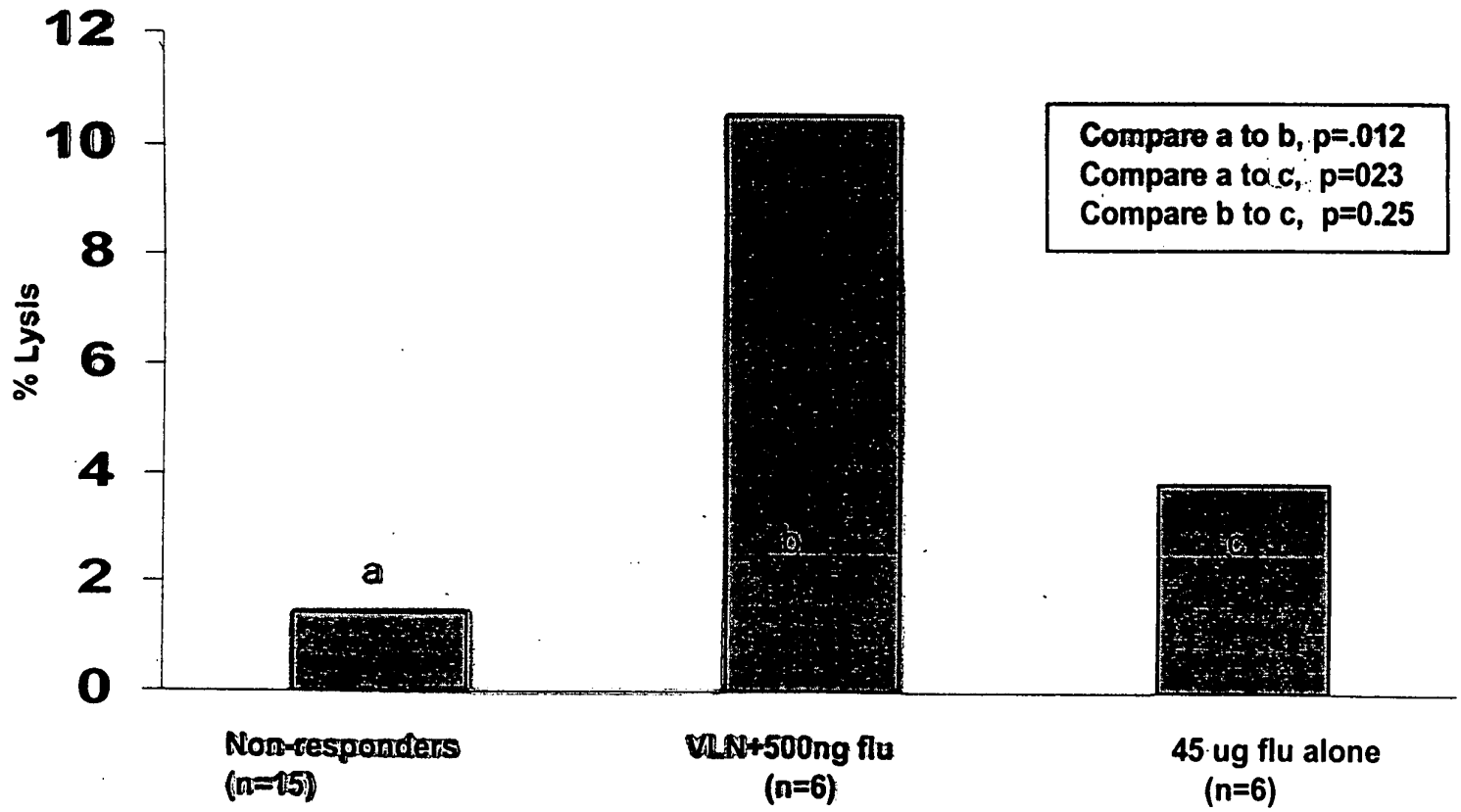
Note: Three start-up companies have been formed around the technologies described in these patents: Warren Pharmaceuticals, Inc; Wellstone Filters, Inc; and VLN, LLC.

1. **Cerami, C.,** Bucala, R., Vlassara, H., Cerami, A. Founds, H.W. *Methods for measurement and treatment predicated on the presence of advanced glycosylation endproducts in tobacco and its combustion byproducts* US patent # 5,850,840 December 22, 1998
2. Cerami A., **Cerami C.,** Ulrich P., *Methods for removing nucleophilic toxins from tobacco smoke.*
3. US Patent #6,119,701 September 19, 2000
4. Cerami A., **Cerami C.,** Ulrich P., *Methods for removing nucleophilic toxins from tobacco smoke.* US Patent #6,615,842 September 9, 2003
5. Brines M., Cerami A., **Cerami C.**, *Protection and enhancement of erythropoietin-responsive cells, tissues and organs.* US Patent #6,531,121 March 11, 2003
6. Gerwien, J., Pedersen, J., Nielsen, J., Kallunki, P., Geist, M., Leist, M., Pederson, L., Bay, K., Brines, M., Cerami, A., **Cerami C.**, Sager, T., Christensen, S. *Recombinant tissue protective cytokines and encoding nucleic acids thereof for protection and enhancement of erythropoietin-responsive cells, tissues and organs.* Filed: 7/01/03, Priority 7/01/02
7. Brines, M., Cerami, A. **Cerami, C.** *Modulation of excitable tissue function by peripherally administered erythropoietin.* Filed: 4/13/00, Priority 4/13/99 (Divisionals: Cerebral ischemia, High dose treatment, transcytosis, neurodegenerative conditions, neuromuscular conditions, enhancement of function)

8. Cerami, A., **Cerami, C.,** Gelber, C., Dove, D., *Methods and devices for modulating the Immune Response.* Filed 4/1/99, Priority 4/1/98

APPENDIX B

CLINICAL TRIAL: VLN IMMUNIZATION INDUCES CTL RESPONSE (DAY 28)



Notes: In order to obtain the statistical information one way analysis of variance (ANOVA) was used.

Source: C. Cunningham-Rundles, 2000, Mount Sinai School of Medicine